REMARKS

Prior to this amendment, claims 1-8, 11-21, and 23-30 are pending. By this amendment, claims 1 and 30 are amended and claims 31-33 are added.

Support for the amendment of claim 1 can be found in the specification at page 8, lines 29- 30 (culture medium comprises substrate concentrations that are similar to those found in the source environment), page 22, lines 26-28 (dilution of sample to density of 1 to 10 cells per ml) and page 23 (detecting growth). Support for the amendment of claim 30 can be found in the specification at page 22, lines 26-28. Support for new claims 31-33 can be found in the specification at page 3, lines 32-33 and at page 19, lines 26-27.

No new matter has been added by this amendment. After entry of this amendment, **claims 1-8, 11-21, and 23-33 are pending**. Unless specifically stated otherwise, none of these amendments are intended to limit the scope of any claim.

Examiner Interview:

Applicants thank Examiner Spiegler for the courtesy of the telephone interview with their representatives Dr. Tanya Harding and Dr. Anne Carlson on August 25, 2004. During the interview, the rejection of the claims under 35 U.S.C. §112 were discussed extensively, as were the rejections under §103. Proposed amendments to the claims, in particular amendments to claim 1 regarding dilution of the sample and detection of growth, were reviewed during the interview. Examiner Spiegler indicated that these proposed amendments were consistent with the language of the specification. Though agreement as to all matters was not reached, it is believed that this Amendment and Response is in accordance with the interview.

Claim objection

Claim 1 was objected to for failing to include a semi-colon following the recitation of "hybridization of a probe to a nucleic acid molecule of the microorganism." Applicants have amended claim 1 to include the semi-colon, and request that this objection be withdrawn.

Claim Rejection Under 35 U.S.C. §112, first paragraph:

Written description

Claims 1-8, 11-21, and 23-30 are rejected for allegedly failing to comply with the written description requirement. Applicants respectfully traverse this rejection.

Claims 1 and 30 are rejected because allegedly there is no support for the recitation "wherein growth of the microorganism comprises an increase in the number of microorganisms in the compartment to no more than about 5 x 10^4 cells/milliliter." Solely in the interest of advancing prosecution of this application, claims 1 and 30 have been amended to remove this phrase, rendering the rejection moot. Applicants respectfully request the withdrawal of this rejection of claims 1 and 30.

As discussed with the Examiner, new phrase "wherein detecting growth of the microorganism consists of detecting an increase in the number of microorganisms in the compartment to no more than about 5 x 10^4 cells/milliliter" has been added to claim 1. This phrase is clearly supported by the specification at Example 4, which describes the detection and quantification of cell growth (see page 23, line 9, through page 24, line 5). Specifically, Example 4 teaches that in order to detect cell growth of microorganisms in a sample, 200 µl (0.2 ml) of cultured microorganisms is aliquoted from the sample and applied to a spot on an array. Furthermore, Example 4 teaches that these spots contain between 100 and 10,000 cells. Thus, given that the volume of the aliquot applied to the spot is 0.2 ml, and that each spot contains between 100 and 10,000 cells, the cell density of the sample ranges between 5 x 10^2 cells/ml and 5 x 10^4 cells/ml (100 cells/0.2 ml and 10,000 cells/0.2 ml, respectively). Thus, the described method can detect the growth of microorganisms up to and including a cell density of 5 x 10^4 cells/ml. Thus, the specification clearly supports the language of claim 1.

Claims 1-8, 11-21, and 23-30 are rejected because allegedly the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor, at the time the application was filed, had possession of the claimed invention. Specifically, claims 1 and 30 are rejected because allegedly the phrase "has

not been cultured using standard culturing techniques" is not adequately described in the specification. Solely in the interest of advancing prosecution of this application, claims 1 and 30 have been amended to remove this phrase, rendering the rejection of these claims moot. Claims 2-8, 11-21 and 23-29 depend, directly or indirectly, from claim 1 and incorporate all of the limitations thereof. Applicants respectfully request that this rejection of claims 1-8, 11-21, and 23-30 be withdrawn.

Enablement

Claims 1-8, 11-21, and 23-30 are rejected for allegedly failing to comply with the enablement requirement. Specifically, the claims are alleged to contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention because the phrase "has not been cultured using standard culturing techniques" allegedly is not adequately enabled by the specification. Applicants respectfully traverse this rejection. However, as described above, claims 1 and 30 have been amended to remove this phrase, rendering this rejection of the claims moot. Claims 2-8, 11-21, and 23-29 depend, directly or indirectly, from claim 1 and include all of the limitations thereof. Applicants respectfully request that this rejection of claims 1-8, 11-21, and 23-30 be withdrawn.

Claim Rejection Under 35 U.S.C. §112, second paragraph:

Claims 1-8, 11-21, 23-30 are rejected as allegedly indefinite over the recitation of "standard culturing techniques" because it is not clear what culturing techniques are considered to be "standard" and what culturing techniques are not considered to be "standard." Applicants traverse this rejection. However, as described above, claims 1 and 30 have been amended to remove the phrase "standard culturing techniques," rendering this rejection of the claims moot. Claims 2-8, 11-21, and 23-29 depend, directly or indirectly, from claim 1 and include all of the limitations thereof. Applicants respectfully request that this rejection of claims 1-8, 11-21, and 23-30 be withdrawn.

Claim Rejection Under 35 U.S.C. §103:

Jovanovich et al. *in view of Sosnowski* et al.

Claims 1-8, 11-21, and 23-25 and 27-29 are rejected as allegedly rendered obvious by Jovanovich *et al.* (USPN 5,756,304) in view of Sosnowski *et al.* (USPN 6,051,380). Applicants traverse this rejection, as these documents, even in combination, do not teach or fairly suggest all of the elements of the claimed invention.

Applicants teach a method of optimizing the growth of a broad spectrum of the organisms in a sample by i) "incubating the microorganism in the culture medium for a period of time and in an environment sufficient to result in growth of the microorganism," wherein the "culture medium comprises substrate concentrations that are similar to those of the source environment," and ii) detecting growth of the microorganism by "detecting an increase in the number of microorganisms in the compartment to no more than about 5 x 10^4 cells/milliliter." This results in Applicants' surprising ability to isolate a wide variety of microoganisms that others of skill in the art have never before been successful at culturing, either because the cell densities are too low (10^3 cells/ml) to detect using known detection methods (for example, optical density), or those of skill in the art did not appreciate the importance of culturing the microorganisms under conditions that are reflective of their natural environment. In contrast, Jovanovich *et al.* teaches a method of isolating microorganisms by actively selecting for *a subset* of microorganisms present in a sample that can be used for bioremediation. Specifically, the reference teaches a method whereby a subset of microorganisms is selected if they survive and/or adapt to selective pressures when cultured in the presence of a toxic compound, such as chromium. Thus, the cultures obtained using Applicants' methods more closely reflect the natural environment, in both the variety and concentration of cells detected, than could be achieved by the methods taught in Jovanovich *et al.*

An important difference between Jovanovich *et al.* and Applicants' invention is that whereas Jovanovich *et al.* teaches growing microorganisms in the presence of a chemical compound with the goal of isolating only those microorganisms that are capable of surviving those specific conditions (Col. 13, lines 13-18), Applicants grow the microorganisms under

conditions "that are similar to those of the source environment," thereby optimizing the ability to isolate a broad spectrum of microorganisms found in the sample (see specification at page 8, lines 15-20). In fact, Jovanovich *et al.* teaches exactly the opposite strategy for isolating cells and thereby teaches away from the claimed invention. By teaching the isolation of only specific cell types in a sample, and that standard culture methods are adequate for its methods (see, for instance, Col. 18, lines 20-27; Col. 19, lines 32-33), Jovanovich *et al.* specifically teaches away from the isolation of all possible microorganisms in a sample.

Applicants further note that Jovanovich *et al.* teaches measuring the growth of the microorganisms by measuring the optical density of a sample. Jovanovich *et al.* relies on optical density readings throughout the specification to detect the microorganisms (see, for instance, Col. 20, lines 58-59; Col. 22, lines 32-40; Col. 23, lines 18-20). Optical density readings are too insensitive to detect cell density at levels of 10^4 cells/ml and below (see footnote 11, Exhibit C of Second Preliminary Amendment submitted on May 10, 2002) which is required in Applicants' claims. No other methods are provided for measuring growth of the microorganisms. Thus, Jovanovich *et al.* cannot be appropriately applied to the pending claims and would not render obvious claims 1-8, 11-21, and 23-25 and 27-29.

The second reference (Sosnowski *et al.*) does not teach anything that makes up for the deficiencies of Jovanovich *et al.* Sosnowski *et al.* teaches the use of microarrays for cell sorting assays in order to select specific cell types. For example, Sosnowski *et al.* teaches selecting cells on the basis of cell surface charge, haptens, and antigens (column 63, lines 23-24). Sosnowski *et al.* does not teach methods of growing microorganisms in order to isolate as many different microorganisms as possible from a single sample, nor does it teach methods of detecting an increase in the number of microorganisms in a compartment to no more than about 5×10^4 cells/milliliter. Thus, Sosnowski *et al.* does not overcome the deficiency of Jovanovich *et al.* Moreover, based on the fact that Jovanovich *et al.* teaches away from the claimed invention, Applicants submit that it is not possible to combine these references.

In light of these arguments and the amendments submitted herein, Applicants respectfully request that this rejection be withdrawn.

Jovanovich et al. *in view of Sosnowski* et al. *and Hoover* et al.

Claim 26 is rejected as allegedly rendered obvious by Jovanovich *et al.* (USPN 5,756,304), in view of Sosnowski *et al.* (USPN 6,051,380) and Hoover *et al.* (Bacteriocins of Lactic Acid, Academic Press Inc. pages 23-39, 1993) because it would have been obvious to one of skill in the art to have modified the method of Jovanovich *et al.* and Hoover *et al.* to provide a more effective means of detection using a microtiter plate and a reporter strain. Applicants traverse this rejection.

Claim 26 depends indirectly from claim 1. Thus, before a meaningful combination can be contemplated with regards to claim 26, it must first be possible to combine Jovanovich *et al.* and Sosnowski *et al.* to render claim 1 obvious. Based on the above discussion, Applicants submit that this is not possible and the rejection based on the further combination of these references with Hoover *et al.* is inappropriate.

Even if one were to make the combination, the following issue must be considered. The Examiner states that Hoover *et al.* teaches the use of reporter strains to provide a more effective means of detection of microorganisms. However, Hoover *et al.* does not teach i) "incubating the microorganism in the culture medium for a period of time and in an environment sufficient to result in growth of the microorganism," wherein the "culture medium comprises substrate concentrations that are similar to those of the source environment," and ii) detecting growth of the microorganism by "detecting an increase in the number of microorganisms in the compartment to no more than about 5 x 10^4 cells/milliliter." Thus, Hoover *et al.* does not overcome the deficiency of Jovanovich *et al.* As discussed above, Jovanovich *et al.* teaches away from Applicants' invention and Sosnowski *et al.* does not overcome the deficiency of Jovanovich *et al.* Thus, this combination of references does not suggest all of the limitations of claim 26 and it would not have been obvious to one of skill in the art to have modified Jovanovich *et al.*, Sosnowski *et al.* or Hoover *et al.* to obtain the invention recited in claim 26. Moreover, since Hoover *et al.* (page 29) describes growing producer and indicator cultures on agar plates (and not under environmentally realistic conditions), Hoover *et al.* also teaches away from Applicants' invention. Thus, Hoover *et al.* cannot be appropriately applied to claim 26 and

would not render obvious this claim. Applicants respectfully request withdrawal of this rejection.

Jovanovich et al. *in view of Sosnowski* et al., *Glockner* et al. and *Amann* et al.

Claim 30 is rejected as allegedly rendered obvious by Jovanovich *et al.* (USPN 5,756,304), in view of Sosnowski *et al.* (USPN 6,051,380), Glockner *et al.* (*System. Appl. Microbiol.*, 19:403-406, 1996) and Amann *et al.* (*J. Bacteriology*, 172:762-770, 1990) because it would have been obvious to modify the method of Jovanovich *et al.* and Sosnowski *et al.* to include steps of vacuum filtration and comparative sequencing to generate an assay that reduces contamination and allows for identification of different subtype strains. Applicants traverse this rejection.

Before a meaningful combination can be contemplated with regards to claim 30, it must first be possible to combine Jovanovich *et al.* and Sosnowski *et al.* Since Jovanovich *et al.*, teaches away from the claimed invention and Sosnowski *et al.* does not overcome the deficiency of Jovanovich *et al.*, Applicants submit that this is not possible. Thus, the rejection based on the further combination of these references with Glockner *et al.* and Amann *et al.* is inappropriate.

Even if one were to combine the references, the following issues must be considered. First, Amann *et al.* teaches the culture of cells in a rich culture broth and the monitoring of growth of the cells by optical density. Thus, Amann *et al.* also teaches away from Applicants' invention, which requires i) "incubating the microorganism in the culture medium for a period of time and in an environment sufficient to result in growth of the microorganism," wherein the "culture medium comprises substrate concentrations that are similar to those of the source environment," and ii) detecting growth of the microorganism by "detecting an increase in the number of microorganisms in the compartment to no more than about 5×10^4 cells/milliliter." Applicants submit that this reference cannot be appropriately applied to claim 30 and the rejection based on the further combination of Jovanovich *et al.*, Sosnowski *et al.*, and Amann *et al.* with Glockner *et al.* is inappropriate.

Second, the Examiner states that Glockner *et al.* teaches "fluorescent in situ detection of rRNA, following vacuum filtration, increases the sensitivity of bacterial detection." However, there is no teaching in Glockner *et al.* to "provide a culture medium based on sea water" to the microorganisms followed by the incubation of the "microorganisms in the medium for a period of time and in an environment sufficient to result in growth of the microorganism," and the detection of "an increase in the number of microorganisms in the compartment to no more than about 5 x 10^4 cells/milliliter," as required by claim 30. Thus, Glockner *et al.* does not overcome the deficiencies of Jovanovich *et al.*, Sosnowski *et al.*, and Amann *et al.* and this combination of references does not teach or suggest all of the limitations of claim 30. In addition, Applicants submit that it would not have been obvious to one of skill in the art to have modified Jovanovich *et al.*, Sosnowski *et al.*, Glockner *et al.* or Amann *et al.* to obtain the invention recited in claim 30. Applicants respectfully request withdrawal of this rejection.

CONCLUSION

Based on the foregoing amendments and arguments, the claims are in condition for allowance and notification to this effect is requested. If for any reason the Examiner believes that a telephone conference would expedite allowance of the claims, please telephone the undersigned at (503) 226-7391.

Respectfully submitted,

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